PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

KATHERINE MOLNAR-KIMBER et al

Appln. No.: **09/576,951** Group Art Unit: **1618**

Filed: **May 24, 2000** Examiner: **Ceperly, M.**

For: **ANTI-RAPAMYCIN MONOCLONAL ANTIBODIES**

DECLARATION OF DR. KATHERINE L. MOLNAR-KIMBER UNDER 37 C.F.R. § 1.132

Assistant Commissioner
of Patents
Washington, D.C. 20231

Sir:

I, Katherine L. Molnar-Kimber do hereby declare and state:

1. I earned my Ph.D. in Immunology from the University of Pennsylvania in 1980, and my studies contributed to the basic understanding of T cell activation and specificity.

2. I received a NIH fellowship from Fox Chase Institute for Cancer Research to study Molecular Virology with Drs. John Taylor and William Mason during 1980-1983, followed by a research associate position. My research elucidated various aspects of the replication cycle of Hepatitis B Virus.

3. After working at Wyeth-Ayerst in the Microbiology and Biotechnology Division on the generation of novel vaccines (1985-1989), I joined the Inflammation/Bone Metabolism Division and characterized the effects of various immunomodulatory agents on the immune system (1989-1995). Thus, in 1993 (at the time of the present invention), I held the position of Research

Scientist in the Inflammation/Bone Metabolism Division of Wyeth-Ayerst Laboratories located in Monmouth Junction, New Jersey, a Division of American Home Products Corporation (AHP).

4. In 1993, I was an invited speaker at Georgetown University and presented a seminar entitled "*Rapamycin as a probe of the immune system*".

5. In 1994, I was an invited speaker at the American Physiological Society, and gave a talk entitled "*Effects of small molecules on cytokine production and responses.*

6. In 1995, I was invited speaker and presented a seminar at the University of Pennsylvania, entitled: "*Mechanism of the Immunosuppressive Activity of Rapamycin*".

7. Also, in 1995, I was an invited speaker at the International Congress on New Immunosuppressive Drugs. International Society of Transplant Surgeons, where I spoke on the Mechanism of Rapamycin's Activity.

8. I am currently a Research Associate Professor in the Department of Pathology & Laboratory Medicine at the University of Pennsylvania School of Medicine.

9. I am a member of the Immunology Graduate Group at University of Pennsylvania, and I give the lecture on "Transplantation" in the first year Immunology Graduate course at the University each year.

10. I am a co-inventor on three patents that describe novel methods for development of ELISA's specific for rapamycins.

11. I am a co-inventor of the patent covering the effector protein of rapamycins.

12. I have co-authored at least 20 articles in the field of immunology.

13. A copy of my *curriculum vitae* is attached hereto.

14. I am a co-inventor of the invention disclosed and claimed in the above-identified application.

15. I have reviewed the Office Action dated August 7, 2001, in the above-identified application, wherein the Examiner, *inter alia*, rejects Claims 33-40 as being unpatentable over Stella et al, Failli et al (A) ('203) and Failli et al (B) ('307), Kao et al ('678) and Kao ('477), Caufield and American Home Products in view of Sevier et al, Yelton et al or Campbell, in further view of Niwa et al.

16. I have also reviewed the references relied upon by the Examiner in the Office Action.

17. In order to demonstrate and establish, *inter alia*, that at the time of the present invention in 1993, there was no reasonable expectation that one could successfully obtain monoclonal antibodies to rapamycins, I further hereby declare and state:

Background to the technology

18. Rapamycins are immunosuppressive agents useful, *inter alia*, in preventing transplant rejection. As with most drugs, the optimal dose to be administered to achieve an optimal level in the blood, and thus the optimal effect *in vivo*, will vary for each individual patient.

19. In order to determine the optimal dose of rapamycins to be administered to a given patient, a means for rapidly determining the amount of rapamycins in the blood is necessary. The present application discloses monoclonal antibodies which are one such means.

20. To facilitate the discussion herein, a brief review of the immune response and antibody production is provided below.

21. The key cells controlling the immune response are T lymphocytes (T cells) and B lymphocytes (B cells). T cells subsequently differentiate into two major types, one of which can activate other cells (helper T cells), and usually expresses the CD4 marker on its surface. The second type of T cell has cytotoxic or regulatory activity (cytotoxic T cells), and usually expresses the CD8 marker on its surface. Antigen presenting cells (APCs), such as macrophages, are also important as they engulf the foreign material bearing antigens, and digest and present the antigens on the cell surface in a form in which the T cells can recognize. Cytokines, which are soluble proteins released by APCs or T cells, such as the interleukins IL-1 to IL-6, also play an important role in signaling between the cells of the immune system.

22. Generally speaking, introduction of a foreign substance, called an antigen, into the body, initiates an immune response which consists of three arms: (a) uptake, digestion and presentation of the antigen by activated macrophages or other APCs, (b) activation of resting T cells that become helper T cells (helper T cells can help activate B cells or they can

activate CD8+ T cells to proliferate and become cytotoxic T cells); and (c) activation of B cells to produce antibody (see Figure 1 attached hereto).

23. T cells can be activated by T cell mitogens or antigen presented in association with histocompatibility antigens, called H-2 in mice or HLA in humans, on the cell surface of APCs.

24. B cells can be activated and induced to differentiate into antibody-secreting cells by interaction with activated helper T cells (called T dependent antigens) or by B cell mitogens (called T cell independent antigens, such as lipopolysaccharide (LPS) or pokeweed mitogen).

25. The induction of antibody production for most antigens utilizes T cell help, and is thus dependent on the activation of T cells and APCs.

26. By 1993, it was well-known that the immune response is initiated upon introduction into the body of a pathogen (such as a virus or bacteria), bearing antigens on its surface. After uptake and presentation by antigen presenting cells, the presence of the antigens is recognized by resting T cells, which coordinate the immune response (see Figure 2 attached hereto, Step I). Following antigen recognition, the T cells become activated (Figure 2, Step II) and produce and release factors called cytokines, in particular, interleukin-2 (IL-2) (Figure 2, Step III). Simultaneously, the T cells express a receptor for IL-2 (the IL-2R). Binding of IL-2 to its receptor on T cells (Figure 2, Step IV) results in T cell proliferation (Figure 2,

Step V). This results in expansion of the population of T cells that recognize and eliminate the specific antigen. This is known as the cellular immune response. Thus, production of IL-2 and expression of IL-2R are necessary steps in the immune response.

27. B cells can also recognize and bind to antigens on the surface of invading pathogens. Generally, in order for the bound B cell to become activated, a further signal is required, which is provided by cytokines (including IL-4, IL-5 and IL-6) released by helper T cells, which have become activated by the process described above. Once activated, the B cells proliferate into plasma cells that produce large amounts of antibodies that are specific for the antigen encountered. These antibodies (also known as immunoglobulins) are secreted into the body fluids, where they encounter and bind to antigens on foreign material, thereby making the foreign material ready for destruction. This is known as the humoral immune response.

28. As of April 1993, immunosuppressive agents were known to suppress the immune response. In April 1993 it was known that such agents can do this by inhibiting any of a number of different steps in the immune response.

The Present Invention

29. The Examiner suggests that at the date of the present invention (April 1993) that generation of monoclonal antibodies to rapamycins would have been obvious to one of ordinary skill in the art because:

(a) it was obvious that the methods disclosed in Sevier et al, Yelton et al and Campbell for preparing monoclonal antibodies to a variety of small drugs could equally be used to prepare monoclonal antibodies to rapamycins; and

(b) it was known that monoclonal antibodies could be generated to FK-506 and, since, natural rapamycin has (according to the Examiner) some structural similarity to FK-506, it was obvious that monoclonal antibodies could also be generated to rapamycins.

30. I do not believe that one of ordinary skill in the art would have considered it obvious or had any reasonable expectation as of April 1993 that monoclonal antibodies to rapamycins could be generated by the methods disclosed in the references cited by the Examiner. There are a number of reasons for this. First, it was known that it was very difficult to generate monoclonal antibodies to immunosuppressive agents since the effective production of antibodies requires a functional immunological system. Secondly, there are significant structural differences between natural rapamycin and FK-506 and these structural differences result in divergent biological activities. Hence, the inhibitory effects of FK-506 and rapamycins on the generation of monoclonal antibodies were expected to be very different. I will discuss these two issues in more detail below.

(a) Generating Monoclonal Antibodies to Immunosuppressants

31. The induction of monoclonal antibodies involves the uptake of antigen, activation of T cells and activation and

differentiation of B cells. Immunosuppressive agents inhibit the immune response and hence, by their very nature, impair the immune response. As described in Niwa et al, "intensive investigations" were performed in order to generate monoclonal antibodies to the immunosuppressive agents, FK-506, inferring that this goal was not straightforward. Likewise, multiple conditions which varied in immunization routes, dosage, compound, schedule and screening methods were investigated in efforts to generate monoclonal antibodies to cyclosporin (CsA), also inferring the difficulty in inducing monoclonal antibodies to the immunosuppressive agent, CsA (Quesniaux et al, *Immunol. Lett.* 9:99-104 (1985); a copy of which is attached hereto). Notably, the references relied upon by the Examiner (Sevier et al, Yelton et al and Campbell) do not disclose or exemplify any monoclonal antibodies to immunosuppressive agents. Accordingly, in April 1993, one of ordinary skill in the art would not have had any reasonable expectation that monoclonal antibodies could be generated to new potent immunosuppressive agents, such as rapamycins. KMK 2/11/02

(b) Differences between FK-506 and rapamycins

32. The structures of FK-506 (also referred to in the art as FR-900506) of Niwa et al and rapamycins may be similar with respect to a portion of the molecules, as shown in brackets in both structures in Figure 3 attached hereto. FK-506 and rapamycins bind to FK-506 Binding Protein (FKBP) at this similar portion, which is known as the FKBP binding domain. However, the remaining portion of the molecules, which is known as the

effector region, is quite distinct. The structural differences between FK-506 and rapamycins are significant because they give rise to differences in biological activity between FK-506 and rapamycins, both at the cellular level and the molecular level. These biological differences are discussed in detail below.

33. At the cellular level, it was known in April 1993 that the mode of action by which rapamycins inhibit the immune response is very different to the mode of action by which FK-506 inhibits the immune response. In particular, it was known that the molecules have:

(i) different effects on the cellular response of the T helper and cytotoxic T cells,

(ii) different effects on an enzyme involved in regulation of the cell cycle of T cells ($p70^{s6}$ kinase),

(iii) different effects on macrophages, and

(iv) different effects on the humoral response involving antibody production by B cells.

Because of these biological differences, one of ordinary skill in the art in April 1993 would not have been able to predict with any reasonable expectation of success, that monoclonal antibodies to rapamycins could have been generated.

34. By April 1993, it was known that rapamycins exert their immunosuppressive effects by inhibiting the proliferative response of T cells to IL-2 (Figure 2, Step IV). In other words, IL-2 is produced, but rapamycins inhibit the ability of IL-2 to continue the progression of the activation of T cells. In contrast, FK-506 acts at an earlier point to inhibit certain

aspects of the activation of T cells in response to antigen (Figure 2, Step II), particularly, those that induce the synthesis of IL-2 (Figure 2, Step III). Thus, in the presence of FK-506, production of IL-2 is blocked. Rapamycins have little effect on either the T cell receptor-induced T cell activation or IL-2 synthesis.

35. It was known by 1993 that, in addition to blocking T cell activation and proliferation at a later stage than FK-506, rapamycins also inhibit the activation of $p70^{s6}$ kinase (Chung et al, *Cell*, 69:1227-1236 (1992); a copy of which is attached). This enzyme is important for the regulation of translation of many proteins important for the progression of the cell cycle of T cells and other cell types. FK-506, on the other hand, does not inhibit the activation of the $p70^{s6}$ kinase (Chung et al, *supra*). Accordingly, these results again illustrate that FK-506 and rapamycins have distinct molecular mechanisms of action.

36. Furthermore, by 1993 it was known that rapamycins and FK-506 have distinct effects on the ability of macrophages to present antigen. As discussed above, this process is an important step in the development of antibodies.

37. More specifically, FK-506 does not affect the accessory phagocytic function of mononuclear cells (Woo et al, *Immunology*, 71:551-555 (1990); a copy of which is attached hereto), but inhibits IL-1 production (Keicho et al, *Cell*

Immunol., 132:285-294 (1991); a copy of which is attached hereto).[1/]

38. On the other hand, my research, the results of which were presented at the Faseb Summer Conference on Autoimmunity in 1991, a copy of the Abstract of which is attached hereto, demonstrated that only at high concentrations did natural rapamycin inhibit IL-1β production from LPS-stimulated monocytes, and at most to a 50% level. These results further indicated in 1993 that the biological activities of rapamycins and FK-506 are quite different, and thus just because monoclonal antibodies to FK-506 were known by 1993 there was no reasonable expectation that one could utilize similar methods to successfully obtain monoclonal antibodies to rapamycins.

39. Finally, rapamycins and FK-506 have very different effects on B cells and antibody production. Although FK-506 blocks T cell receptor (TCR)-induced T cell activation, it does not directly affect B cell antibody production in most models (Stevens et al, *Transpl.*, 51:1240-1244 (1991); and Morikawa et al, *Transpl.*, 54:1025-1030 (1992), a copy of each of which is attached hereto). Although FK-506 inhibits B cell activation when stimulated with anti-IgM and anti-IgM + IL-4, FK-506 does not inhibit B cell activation induced by LPS or by

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[1/] Cooper et al, *Transplant Proc.*, 23(6):2957-2958 (1991), a copy of which is attached hereto, taught that the effects of FK-506 on macrophage function may be complicated by the possibility of drug carryover. However, since Keicho et al, *supra* used a cell line to assess the ability of FK-506 to inhibit IL-1 production, this is not an issue.

8-mercaptoguanosine. The effects of FK-506 on B cell activation parallel results observed with CsA (Wicker et al, *Eur. J. Immunol.*, 20:2277-2283 (1990), a copy of which is attached hereto).

40. In addition, it was known that FK-506 actually augments antibody production in some models (Yamamoto et al, *Immunol.*, 69:222-227 (1990), a copy of which is attached hereto).

41. On the other hand, it was known that rapamycins directly block antibody production from B cells, and also block T cell activation, and can inhibit T-dependent antibody production (Luo et al, *Transpl.*, 53:1071 (1992), a copy of which is attached hereto) via mechanisms distinct from CsA. Rapamycins inhibit B cell activation induced by all stimuli tested, although some effects of the drug result in a delay of the activation (Wicker et al, *supra*). Thus, in 1993, one of ordinary skill in the art would not have had any reasonable expectation that one could successfully obtain monoclonal antibodies to rapamycins, because rapamycins were known to inhibit antibody formation in an entirely different manner than FK-506 and CsA.

42. The many differences in biological activity between rapamycins and FK-506 at the cellular level, described above, are a reflection of the fact that rapamycins and FK-506 also have very different modes of action at the molecular level. It was well understood by April 1993, that although both FK-506 and rapamycins bind to FKBP, they have distinct molecular actions,

as illustrated in detail in Figure 4 attached hereto. That is, by 1993, it was known that after the TCR triggers activation of the T cell, the FK-506-FKBP complex binds to the calmodulin-calcineurin complex and inhibits calcineurin's ability to dephosphorylate the cytoplasmic subunit of NF-AT (O'Keefe et al, *Nature*, 357:692-694 (1992); Clipstone et al, *Nature*, 357:695-697 (1992); and Schreiber et al, *Immunology Today*, 13:136-142 (1992); a copy of each of which is attached hereto). The inhibition of calcineurin activity results in inhibition of the production and secretion of important T cell cytokines, such as IL-2 and other cytokines (Tocci et al, *J. Immunol.*, 143:718-726 (1989); a copy of which is attached hereto). Without the secretion of IL-2 or other cytokines, T cell activation does not proceed (see Figure 4).

43. Rapamycins, on the other hand, bind to FKBP but then the rapamycin-FKBP complex does not bind to the calmodulin-calcineurin complex (Fruman et al, *Proc. Natl. Acad. Sci. USA*, 89:3686-3690 (1992); a copy of which is attached hereto). This was known in April 1993, and many groups were pursuing the identification of the unique molecule to which the rapamycin-FKBP complex binds. The molecule was later identified by our group at Wyeth-Ayerst and by three other groups, and referred to by various names, including FRAP, RAFT1, SEP and mTOR (Brown et al, *Nature*, 369:756-758 (1994); Sabatini et al, *Cell*, 78:35-43 (1994); Chen et al, *BBRC*, 203:1-7 (1994); and Sabers et al, *J. Biol. Chem.*, 270:815-822 (1995); a copy of each of which is attached hereto).

44. Rapamycins can inhibit 50% of the activation of T cells via the CD28 molecule, which binds the B7 molecule, found on APCs (Kay et al, *Immunol.*, 72:544-549 (1991); and Sigal et al, *Transplant. Proc.*, 23(2)Suppl.2:1-5 (1991); a copy of each of which is attached hereto). Blockage of stimulation via the CD28-B7 pathway results in inhibition of antibody production (Linsley et al, *Science*, 257:792-795 (1992); a copy of which is attached hereto). CsA and FK-506 are unable to block activation of T cells via the CD28 pathway (Sigal et al, *supra*). Thus, it was believed in 1993 that rapamycins would be considerably more efficient at blocking antibody production *in vivo* than FK-506.

45. In the light of the above, it is clear that the biological activities of rapamycins and FK-506 were known in 1993 to be ~~completely~~ significantly KMK 2/11/02 different, both at the cellular and the molecular level. Accordingly, the generation of monoclonal antibodies to FK-506 in 1993 was of no predictive value as to whether monoclonal antibodies to rapamycins would be able to be generated. Furthermore, one of ordinary skill in the art would not have considered that the methods for generating monoclonal antibodies to FK-506 of Niwa et al, when applied to rapamycins would result in the generation of monoclonal antibodies to rapamycins. It is worth noting that FK-506 and CsA, on the other hand, exhibit very similar biological activities. The inhibitory effects of FK-506 against T cells, B cells and APC parallel those of CsA. FK-506 and CsA immunophillin complexes inhibit the same molecular target, i.e., calcineurin. Thus, since monoclonal antibodies have been induced towards CsA

(Quesniaux et al, *supra*), it would have been expected that one could induce monclonal antibodies specific for FK-506. In contrast, since rapamycins inhibit T cell activation at a later stage, suppress antibody production from B cells *per se*, and modulate the activity of distinct molecular targets (p70s6 kinase, effector protein), one of ordinary skill in the art would not have any reasonable expectation of success in generating monoclonal antibodies to rapamycins, even in light of the teaching of Niwa et al.

46. In summary, the production of monoclonal antibodies involves the activation of APCs and presentation of the antigen, activation of T cells and activation and differentiation of B cells. In April 1993 it was known that it is difficult to generate monoclonal antibodies to immunosuppressive agents. Since in April 1993, rapamycins were known to be potent immunosuppressive agents, one of ordinary skill in the art would not have had any reasonable expectation of success in generating monoclonal antibodies to rapamycins.

47. The fact that monoclonal antibodies had, with some difficulty, been generated to FK-506 does not change this. There are significant structural differences between FK-506 and rapamycins, and these result in divergent biological activities.

48. At the cellular level, rapamycins and FK-506 differ in their effects on:

(i) the cellular response of T cells,

(ii) an enzyme involved in regulation of the cell cycle of T cells,

(iii) activated macrophages, and

(iv) the humoral response, involving antibody production by B cells.

49. At the molecular level, rapamycins and FK-506 differ in the effector proteins to which they bind and the molecular pathways which they target.

50. Accordingly, rapamycins have a significantly different effect on the immune system and, in particular, on antibody production, compared to FK-506. Thus, one of ordinary skill in the art in April 1993 would not have reasonably predicted based on the teachings in Niwa et al that monoclonal antibodies to rapamycins could have been generated.

Conclusions

In view of the foregoing, it is evident that at the time of the present invention in 1993, there clearly was no reasonable expectation that one could successfully obtain monoclonal antibodies to rapamycins, as claimed in the present application.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

DECLARATION OF DR. KATHERINE L. MOLNAR-KIMBER
UNDER 37 C.F.R. § 1.132
U.S. Appln. No. 09/576,952

Date: Feb 11, 2002

Name: Katherine L. Molnar-Kimber
Katherine L. Molnar-Kimber